UNIVERSITY OF PENNSYLVANIA

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IN PHYSIOLOGICAL CHEMISTRY

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PARTIAL FULFILMENT OF THE REQUIREMENTS FOR
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Reprint from
*The Journal of Biological Chemistry*, Vol. LXXXVIII, No. 2

PHILADELPHIA
1930

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# THE APPLICATION OF THE QUINHYDRONE ELECTRODE TO THE DETERMINATION OF THE pH OF SERUM AND PLASMA.*

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(Received for publication, May 24, 1930.)

## INTRODUCTION.

The need for an accurate method for determining the pH of small amounts of biological fluids with a minimum expenditure of time has resulted in considerable search for a substitute for the hydrogen electrode. Colorimetric methods for determining the pH of serum and plasma were especially devised to meet this need (1, 2). But several investigators (3–5) have been unable to obtain reliable results with the colorimetric procedure, and the methods have come to be regarded as insufficiently accurate for careful experimental work. The quinhydrone electrode seemed worthy of consideration. Although its use in whole blood is still a mooted question, most investigators agree that it can be used successfully with human serum and plasma. In view of the fact that the dog is so widely used as an experimental animal, we were prompted to make a critical study of the applicability of this method to dog serum. 38° was selected as the working temperature since all conversions of the pH of serum from 20–38° are subject to a temperature correction which on theoretical grounds alone should not be regarded as constant under a variety of experimental conditions.

* This paper constitutes a thesis submitted to the Graduate School of the University of Pennsylvania in partial fulfilment of the requirements for the degree of Doctor of Philosophy. The investigation was carried out under the direction of Dr. D. Wright Wilson.

*Theoretical.*

The theory of oxidation-reduction potentials and their relation to pH has been discussed many times by different writers. The reader is therefore referred to the excellent reviews on the subject by Clark (6, 7) and by Biilmann (8). In acid or weakly alkaline solutions, the simplified equation for the quinhydrone electrode is as follows:

$$E_h = E_Q + \frac{RT}{F} \ln [H^+] \quad (1)$$

where $E_Q$ is the characteristic constant of the quinhydrone system expressed in volts. At 20° the value is 0.7029 and at 38° 0.6896 (Clark (6)). We were able to duplicate these values repeatedly in standardizations of the electrode by means of buffer solutions.

Blood serum is just alkaline enough to be slightly beyond the range where the simplified equation holds, but it is possible to apply a correction factor, $E_c$. The modified equation becomes:

$$E_h = E_Q + E_c + \frac{RT}{F} \ln [H^+] \quad (2)$$

Thus, while $E_c$ remains zero in acid solution, in going up the pH scale beyond 7.0 it assumes a gradually increasing positive value. The point on the pH scale where this occurs is shifted by the temperature at which the electrode is used. For example, at 20° in a 0.2 M phosphate buffer solution, the point where $E_c$ begins to be appreciable in value is about pH 7.6; at 38° the point is about pH 7.0. It becomes at once apparent that at 20° it is possible to determine the pH of most biological fluids with the quinhydrone electrode on the assumption that $E_c$ remains zero (9, 10). At 38°, however, this assumption cannot be made. Since $E_c$ is a function of the pH it was determined experimentally at 20° and 38° in the range 7.0 to 8.0 with a series of phosphate buffers of known pH. The results of these standardizations are the two curves shown in Fig. 1 (*cf.* La Mer and Parsons (11)).

*Calculation of pH from Quinhydrone Electrode Readings.*— Routine calculations are made by the use of the following equation derived from Equation 2.

$$\text{pH} = \frac{E_Q - (\text{E.M.F.} + e_{\text{sat. cal.}})}{0.0001983\ T} + \frac{E_c}{0.0001983\ T} \quad (3)$$

where E.M.F. represents the observed voltage, and (E.M.F. + $e_{sat.\ cal.}$) = $E_h$. The above equation may serve at any temperature, provided the proper values of $E_Q$ and $e_{sat.\ cal.}$ are used. If the pH is to be determined within the range where $E_c$ is appreciable, it has been customary to proceed as follows: (1) Calculate the approximate pH of the solution with the above equation, $E_c$ being assumed to be zero. (2) From one of the curves in Fig. 1 read off $E_c$ corresponding to this pH. (3) Add to the approximate pH this value of $E_c$ (expressed in pH units). The pH thus determined is assumed to represent the pH of the solution.

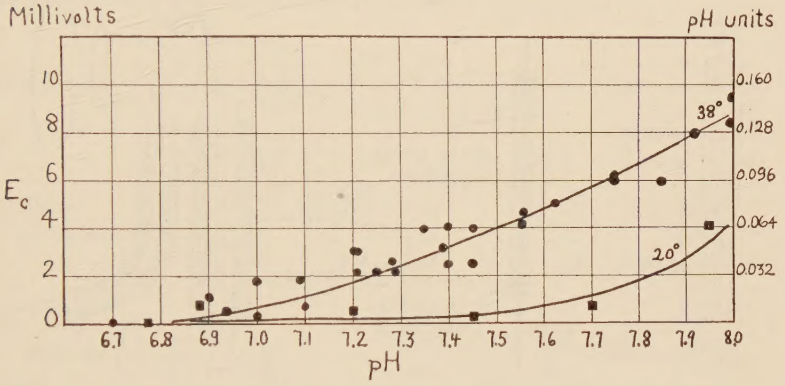


FIG. 1. The variation of $E_c$ with pH at 20° and 38°. pH units (ordinate) = $\frac{E_c}{0.000198T}$.

*Apparatus.*

The hydrogen electrode apparatus and accessory equipment were standard. The chambers were the Clark-Cullen 2 cc. rocking type. The saturated calomel half-cell was the standard reference cell. Duplicate set-ups were available for simultaneous determinations at 20° and 38°.

For the quinhydrone electrode, special micro capillary chambers of Pyrex glass, having a capacity of about 0.1 cc., were devised. These chambers consisted of a barrel large enough to admit the glass tube carrying the electrode, and an accurately centered capillary 25 mm. long and 1.5 mm. in diameter. The end of the

capillary was constricted somewhat to diminish diffusion of KCl into the capillary, and to keep solid quinhydrone from dropping out. The electrodes of 1 mm. gold or platinum wire were 20 mm. long and sealed with special glass into glass tubing. The lead was soldered directly to the electrode. No mercury connections were used. When the electrode was put into the chamber, the end was about 10 mm. from the end of the capillary. Fig. 2 shows a sketch

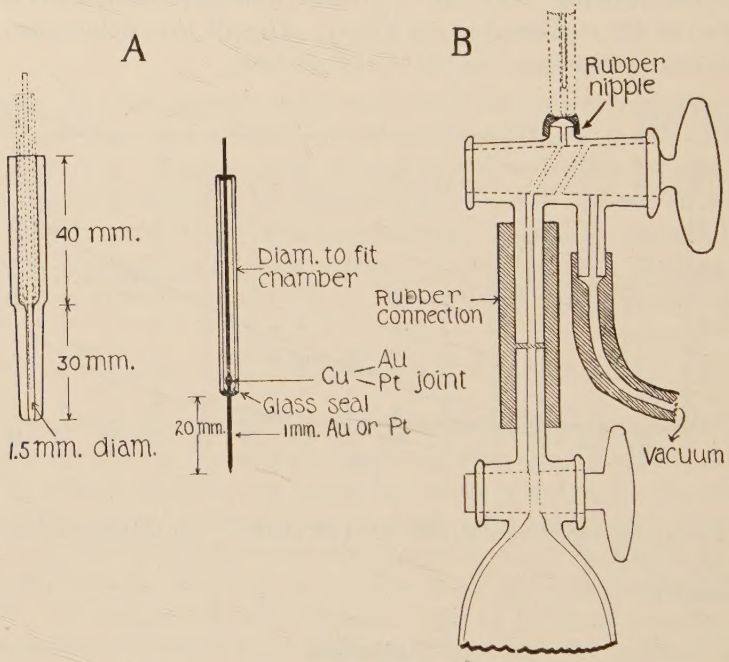


FIG. 2. *A*, details of the quinhydrone electrode and capillary chamber; *B*, filling device.

of the chamber and the electrode. The chambers were filled by forcing the liquid up into the capillary from a tonometer by means of a mercury leveling bulb. The device for accomplishing this without loss of $CO_2$ is shown in Fig. 2. The electrode and vessel are similar in general design to those recently described by Gesell (12) who also makes use of an auxiliary device for forcing the liquid into the chambers. We feel that the use of a plunger type described by Cullen (13) where liquid fills the chamber due to

decreased pressure within the chamber may cause loss of $CO_2$. In certain poorly buffered fluids like pancreatic juice, the effect of such loss of $CO_2$ has been shown to occur

*Methods.*

The hydrogen electrode analyses were standardized with 0.1 N HCl, a pH of 1.076 at 20° and 38° being assumed, and $e$ for the entire reference system consisting of the saturated calomel cell plus liquid junction potential was calculated to be 0.2358 volt at 38° and 0.2475 volt at 20°. Hydrogen electrode determinations of serum were made with the Hasselbalch technique.

TABLE I.

*Comparison of pH of Artificial Serum As Determined by Hydrogen and Quinhydrone Electrodes at 38°.*

| | Experiment No. | $pH_{H_2}$ | $pH_Q$ | $pH_{H_2} - pH_Q$ |
|---|---|---|---|---|
| Buffered with phosphate. | 1 | 7.414 | 7.39 | +0.02 |
| | 2 | 7.512 | 7.52 | −0.01 |
| | 3 | 7.380 | 7.35 | +0.03 |
| Unbuffered. | 1 | 7.462 | 7.34 | +0.12 |
| | 2 | 7.574 | 7.50 | +0.07 |
| | 3 | 7.475 | 7.42 | +0.06 |

The electrodes for the quinhydrone determinations were always polished on cork with fine emery before use. After being used on serum, they were put overnight in cleaning solution and then for a day in distilled water. The C.P. platinum electrodes advised by Cullen (13) were found to be no better than those made from ordinary platinum wire. In regard to the use of gas flames in the preparation of the electrodes, we have not detected any poisoning such as might be revealed by erratic behavior.

In making quinhydrone determinations, the following procedure was adopted. Dry quinhydrone was dusted into the capillary chamber with a medicine dropper, care being taken to get approximately the same amount into each. Then the electrode was inserted and connection made to the lead-off wire. In order to prevent waste of material and loss of $CO_2$ in transfer, the stopcock (Fig. 2, *B*) with rubber nipple to accommodate the electrode

chamber was fitted to the tonometer containing the serum. After the system was filled with serum and a small amount allowed to run out, the apparatus was ready for use. When a quinhydrone chamber was put in place, the stop-cock was opened slowly and serum allowed to flow into the capillary. The serum was thus forced up beyond the glass seal of the electrode. As soon as the chamber was filled it was suspended in a saturated KCl bridge, so as just to touch the surface of the KCl, and the voltage read on the potentiometer. Excess serum remaining in the nipple and dead space below was sucked off by vacuum on turning the stop-cock through 180°. The electrode was tested for the effect of KCl diffusion and loss of $CO_2$. No noticeable diffusion of KCl into the capillary occurred within 10 minutes, which is ample time for any determination. Loss of $CO_2$ was studied with an "artificial serum" equilibrated at 40 mm. of $CO_2$ tension, consisting of 0.082 M NaCl, 0.022 M $NaHCO_3$, and, where buffer was desired, 0.055 M $NaH_2PO_4$ to give it the buffer capacity of serum. Comparative determinations by the hydrogen and quinhydrone electrodes were made. Table I gives the results of six such experiments. The results with the quinhydrone electrode used on solutions having the buffer capacity of serum checked with those of the hydrogen electrode quite closely. This was not the case, however, with the unbuffered artificial serum.

Fig. 3 shows the behavior of the quinhydrone electrode in 0.2 M phosphate buffers, buffered artificial serum, and unbuffered artificial serum. Although the drift of the electrode in buffered artificial serum is noticeable, and goes in an alkaline direction, which might indicate some loss of $CO_2$, this is nevertheless slight and insignificant when compared with a drift in blood serum due to other causes. But in the unbuffered artificial serum, the electrode exhibits not only an unmistakable drift due apparently to loss of $CO_2$, but also very erratic behavior. This confirms the findings of Cullen and Earle (9) and Kolthoff (14, 15).

For the experiments described below, blood was drawn from dogs by heart puncture and collected under oil. If serum was desired, the blood was allowed to clot in centrifuge tubes; if plasma, enough of a concentrated solution of $K_2C_2O_4$ and NaF 3:1 was added to make a 0.4 per cent solution of the mixed salts. It is a curious fact that hemolysis of dog blood seldom occurs when

a concentrated solution of anticoagulant is used, while some hemolysis nearly always is evident when the dry salts are mixed with the blood. The blood was centrifuged under solid paraffin for 20 minutes. The supernatant serum or plasma was drawn off and stored in tonometers over mercury. Transfer was made directly from the tonometers into the hydrogen and quinhydrone electrode chambers.

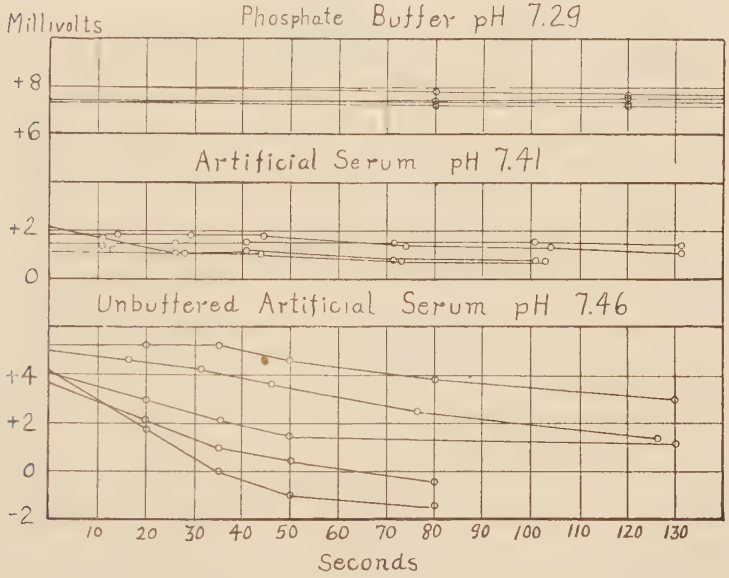


FIG. 3. The drift of the quinhydrone electrode in certain solutions of inorganic salts.

## EXPERIMENTAL.

### *Use of Quinhydrone Electrode on Serum.*

In attempting to determine the pH of serum and plasma with the quinhydrone electrode at 38°, a striking fact presented itself. As soon as the quinhydrone was mixed with serum, a very pronounced drift toward more acid pH occurred which was accompanied by the formation of a pale to deep red color in the capillary. This drift was presumed to be due to a side reaction whereby some

constituent of the serum, possibly the protein, combined either with oxidant or reductant. Reimers (16) in some test-tube experiments has shown that a reaction producing a reddish brown precipitate occurs with most protein solutions and quinone, but not

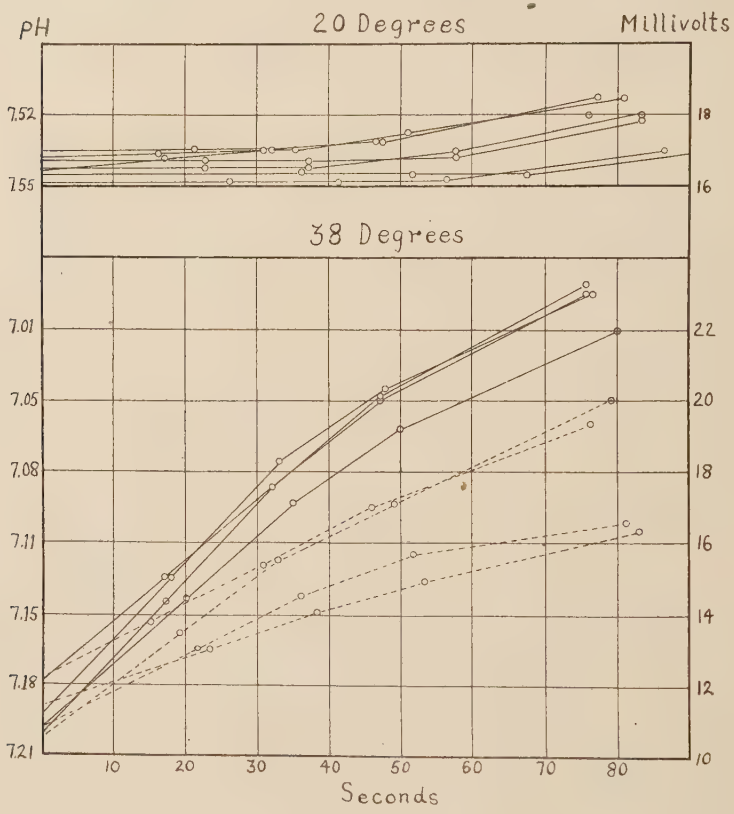


Fig. 4. The drift of the quinhydrone electrode in dog serum at 20° and 38°.

hydroquinone. In addition it was observed that the rate of drift and the degree of color formation were directly proportional to the excess amount of quinhydrone added to the capillary. If the excess quinhydrone over the amount necessary to saturate the serum was great, the drift was increased; if the excess was small, the drift was much reduced. In Fig. 4 are shown the drifts of the

quinhydrone electrode in serum at 38° and 20°. The broken lines represent determinations where the excess of quinhydrone was slight, while the solid lines represent cases where larger amounts were used. Whereas the drift at 38° varies from 0.02 to 0.04 pH in 15 seconds, to 0.2 pH in 1 minute, at 20° the observed voltage sometimes remains constant over a period of 30 to 40 seconds before an acid drift begins. It becomes obvious from these results why previous investigators have made observations at room temperature.

Because of the great drift at 38°, no reliable determinations could be made unless the time relations were closely observed. This necessitated special technique. As soon as the serum was forced up into the capillary from the tonometer to mix with the quinhydrone, a stop-watch was started and the electrode suspended in the KCl bridge. Then a reading on the potentiometer was made as rapidly as possible. This interval of time usually averaged 15 seconds, with a single operator. Two more readings were made at 15 seconds intervals, and frequently a third 30 seconds later. By plotting the time against the voltage reading, the lines shown on the graph were obtained. In order to approximate the voltage of the electrode at the moment when contact of the quinhydrone with the serum first occurred, extrapolation to zero time was resorted to. It is to be emphasized that extrapolation to zero along straight lines is probably not entirely correct, but the fact remains that too little is known about the behavior of the electrode in the first few seconds after the quinhydrone has come in contact with the serum to make a mathematical curve extrapolation justifiable. The extrapolated values usually ranged from 0.02 to 0.04 pH more alkaline than the first reading at 15 seconds after mixing, and were assumed to represent the pH of the serum before any complicating side reaction could occur. The lines extrapolated toward zero time always converged. The scattering of the extrapolated values was 0.04 pH. Deviations from the average readings of six or seven different electrodes were rarely greater than 0.02 pH. In fact, a series always grouped itself so that when occasional single erratic results did occur, they could be ignored. It must be emphasized, however, that in a system where such violent drifts do occur, single determinations are of doubtful value. On this account usually from four to six determinations

were made and occasionally from ten to twelve. When one is working in this way, each comparison of the pH of serum by the hydrogen and quinhydrone electrode represents two hydrogen electrode values which in 90 per cent of the cases checked each other within 0.01 pH, and generally five or six quinhydrone determinations. The difference between the average values obtained by the two methods was taken to represent the deviation of the

TABLE II.

*Typical Experiments Which Illustrate Comparative pH Determinations of Dog Serum at 38° by Hydrogen and Quinhydrone Electrodes.*

| Dog No. | Date. | Hydrogen electrode. | | Quinhydrone electrode. | | | | $pH_{H_2} - pH_Q$ |
|---|---|---|---|---|---|---|---|---|
| | | pH | Average pH. | Electrode No. | pH | | Average pH. | |
| | *1929* | | | | | | | |
| 7 | Aug. 5 | 7.390 | | Au 1 | 7.33 | 7.34 | | |
| | | 7.387 | 7.389 | Au 6 | 7.35 | 7.36 | | |
| | | | | Au 2 | 7.34 | 7.33 | | |
| | | | | Pt 5 | 7.34 | | | |
| | | | | Pt X | 7.34 | | 7.34 | +0.05 |
| 8 | Aug. 6 | 7.321 | | Au 8 | 7.29 | 7.28 | | |
| | | 7.324 | 7.322 | Au 7 | 7.29 | | | |
| | | | | Au 3 | 7.29 | 7.29 | | |
| | | | | Pt 3 | 7.29 | | | |
| | | | | Pt 1 | 7.30 | | 7.29 | +0.03 |
| 2 | July 18 | 7.279 | | Au 6 | 7.27 | 7.27 | | |
| | | 7.279 | 7.279 | Au 5 | 7.28 | 7.29 | | |
| | | | | Au 1 | 7.25 | 7.28 | | |
| | | | | Au 8 | 7.25 | 7.27 | 7.27 | +0.01 |

quinhydrone electrode reading from the true pH of the serum. Table II represents several typical comparisons. The individual quinhydrone electrodes of gold or platinum are designated by numbers. All pH calculations for the quinhydrone electrode reported in this paper were made from potentials extrapolated to zero time.

Table III shows comparative data on the pH of dog serum and plasma of normal dogs by the hydrogen and quinhydrone electrodes. Except in one isolated case, the quinhydrone electrode

TABLE III.

*Comparison of pH of Serum and Plasma of Normal Dogs at 38° by Hydrogen and Quinhydrone Electrodes.*

| Dog No. | Date. | $pH_{H_2}$ | $pH_Q$ | $pH_{H_2} - pH_Q$ | Remarks. |
|---|---|---|---|---|---|
| | *1929* | | | | |
| 2 | July 3 | 7.336 | 7.32 | +0.02* | Animal pregnant. |
| | " 10 | 7.239 | 7.20 | +0.04 | |
| | " 18 | 7.305 | 7.27 | +0.04 | |
| 3 | " 11 | 7.322 | 7.27 | +0.05 | Slight hemolysis. Wk. after |
| | Aug. 7 | 7.340 | 7.28 | +0.06 | injection of NaCN. |
| | " 14 | 7.306 | 7.30 | +0.01 | |
| 4 | July 12 | 7.336 | 7.31 | +0.03 | |
| 6 | " 17 | 7.361 | 7.35 | +0.01 | Slight hemolysis. |
| | " 24 | 7.368 | 7.36 | +0.01 | |
| 7 | Aug. 5 | 7.389 | 7.34 | +0.05 | |
| | " 9 | 7.354 | 7.30 | +0.05 | NaF added to serum. |
| 8 | " 6 | 7.321 | 7.29 | +0.03 | " " " " |
| 11 | Nov 1 | 7.351 | 7.30 | +0.05 | |
| | " 15 | 7.410 | 7.36 | +0.05 | |
| | *1930* | | | | |
| | Jan. 16 | 7.368 | 7.31 | +0.06 | |
| | Feb. 19 | 7.388 | 7.35 | +0.04 | 48 hrs. after 1 per cent hemor- |
| | Apr. 4 | 7.378 | 7.32 | +0.06 | rhage. |
| | *1929* | | | | |
| 12 | Dec. 27 | 7.323 | 7.26 | +0.06 | Blood kept at 38° 35 min. |
| | " 13 | 7.385 | 7.32 | +0.06 | before centrifuging. |
| | *1930* | | | | |
| | Feb. 14 | 7.381 | 7.36 | +0.02 | |
| | " 15 | 7.425 | 7.42 | +0.01 | Mixture of serum and plasma. |
| 15 | Jan. 24 | 7.358 | 7.34 | +0.02 | |
| | Feb. 24 | 7.353 | 7.34 | +0.01 | |
| 16 | " 12 | 7.390 | 7.38 | +0.01 | Mixture of serum and plasma. |
| | " 12 | 7.363 | 7.35 | +0.01 | Plasma. |
| | " 25 | 7.432 | 7.44 | −0.01 | " |
| 17 | Mar. 4 | 7.400 | 7.38 | +0.02 | " on ice overnight. |
| | " 5 | 7.455 | 7.44 | +0.02 | " |
| | Apr. 17 | 7.352 | 7.32 | +0.03 | |
| 18 | Mar. 6 | 7.480 | 7.47 | +0.01 | Plasma. |
| Average..... | | | | +0.03 | |

* Serum was used unless otherwise specified.

always gave pH values which were more acid than those obtained by the hydrogen electrode. We have chosen to call this difference between the hydrogen and quinhydrone pH values, the *Q* correction throughout the remainder of the paper. The data are arranged to show variation of this correction in individual animals. In thirty-six normal samples from twelve dogs (see also Tables VII and VIII) the *Q* correction averaged +0.03 pH with a maximum spread extending over 0.08 pH. If we assign +0.03 pH as the constant correction of the quinhydrone electrode for normal dogs, then 78 per cent of all the determinations check the hydrogen electrode within ±0.02 pH and 95 per cent within ± 0.03 pH.

*Effect of Hemorrhage or Injection of Sodium Cyanide.*

In view of the experience in this laboratory with the colorimetric method on dog serum and plasma after hemorrhage (3, 5), some experiments were carried out to see whether hemorrhage or injection of NaCN would cause a variation in the *Q* correction. Gesell and Hertzman (17) in their continuous recording experiments on the pH of dog blood with the manganese dioxide electrode, found after hemorrhage, and especially after injection of 0.01 M NaCN, that the $MnO_2$ electrodes became erratic, but in general, the quinhydrone electrodes agreed with the hydrogen electrode values within 0.05 pH or less. Only occasionally was a larger deviation observed.

If hemorrhage affects the quinhydrone electrode, it may do so in two ways: (1) by lowering the protein concentration through dilution which in turn decreases the buffer capacity of serum; (2) by piling up metabolites resulting from insufficient oxidation. These two effects do not occur simultaneously. The protein concentration does not reach its lowest ebb until 18 to 24 hours after the hemorrhage, when influx of tissue fluids low in proteins has restored the blood volume. Metabolites, on the other hand, pile up rapidly, so that the blood may become more acid than normal within 20 to 30 minutes after hemorrhage. Whether the magnitude of the acid change is a direct measure of the quantity of metabolites present has not been shown, although such a presumption appears probable.

In the five experiments, in Table IV, the effect of hemorrhage on the *Q* correction was studied. In no case was there a change in the

$Q$ correction sufficiently great to be attributed to the immediate effects of the hemorrhage. 24 hours after hemorrhage, when the colorimetric corrections always ran low, it was thought that the $Q$ correction might be high due to diminution of the buffer capacity

TABLE IV.

*Effect of Hemorrhage on Quinhydrone Correction.*

| | Date. | $pH_{H_2}$ | $pH_Q$ | $pH_{H_2} - pH_Q$ | Remarks. |
|---|---|---|---|---|---|
| | *1929* | | | | |
| Dog 2, 16.8 kilos. | July 18 | 7.305 | 7.27 | +0.04 | Normal. |
| | | 7.279 | 7.27 | +0.01 | 80 min. after 2.6 per cent hemorrhage.* |
| | " 19 | 7.309 | 7.23 | +0.08 | 24 hrs. after 3.1 per cent hemorrhage. |
| Dog 6, 6.7 kilos. | July 24 | 7.368 | 7.36 | +0.01 | Normal. |
| | | 7.380 | 7.35 | +0.03 | 130 min. after 1.9 per cent hemorrhage. |
| | " 25 | 7.390 | 7.38 | +0.01 | 24 hrs. after 3.2 per cent hemorrhage. |
| Dog 7, 18.2 kilos. | Aug. 9 | 7.354 | 7.30 | +0.05 | Normal. |
| | | 7.297 | 7.25 | +0.05 | 20 min. after 3.3 per cent hemorrhage. |
| | " 12 | 7.331 | 7.32 | +0.01 | 3 days after hemorrhage. |
| | *1930* | | | | |
| Dog 15, 19.7 kilos. | Jan. 24 | 7.358 | 7.34 | +0.02 | Normal. |
| | " 25 | 7.438 | 7.41 | +0.04 | 24 hrs. after 3 per cent hemorrhage. |
| | " 29 | 7.382 | 7.36 | +0.02 | 4 days after hemorrhage. |
| Dog 17, 14 kilos. | Apr. 17 | 7.352 | 7.32 | +0.03 | Normal. |
| | | 7.260 | 7.20 | +0.06 | 30 min. after 3.5 per cent hemorrhage. |
| | " 18 | 7.408 | 7.36 | +0.05 | 24 hrs. after. 4 per cent hemorrhage. |

* Amounts of blood removed are expressed as per cent of body weight.

of the serum. Here again, no effect on the $Q$ correction was noted except in the case of Dog 2.

The immediate effect of hemorrhage on the occurrence of metabolites in the blood stream may possibly be correlated with the effect of NaCN injection. In this case also, oxidation is interfered with. Usually the blood becomes more acid very soon after the injection of 0.01 N NaCN. Since only two of our experiments

listed in Table V show a small acid change, it is not certain to what extent metabolites may have piled up. On the other hand, since we observed more or less severe hyperpnea in all dogs injected, and regarded it as a satisfactory indication of the NaCN effect, this may have somewhat masked the acid swing. In two experiments, the injection of NaCN did not affect the $Q$ correction. In the last experiment (Dog 3), however, a change in the $Q$ correction appeared. The fact that in this determination the hydrogen electrode gave somewhat erratic results does not allow us to place too much weight on the experiment.

TABLE V.

*Effect of Injection of Sodium Cyanide on Quinhydrone Correction.*

| | Date. | $pH_{H_2}$ | $pH_Q$ | $pH_{H_2} - pH_Q$ | Remarks. |
|---|---|---|---|---|---|
| | *1929* | | | | |
| Dog 7, 18.2 kilos. | Aug. 5 | 7.389 | 7.34 | +0.05 | Normal. |
| | | 7.343 | 7.33 | +0.01 | 5½ min. after injection of 18 cc. 0.01 M NaCN. |
| Dog 8, 17.7 kilos. | Aug. 6 | 7.321 | 7.29 | +0.03 | Normal. |
| | | 7.331 | 7.29 | +0.04 | 72 sec. after injection of 15 cc. 0.01 M NaCN. |
| Dog 3, 11.6 kilos. | Aug. 7 | 7.340 | 7.28 | +0.06 | Normal. |
| | | 7.31* | 7.19 | +0.12 | 45 sec. after injection of 14.5 cc. 0.01 M NaCN. Convulsions. |

* Single determination.

*Effect of Allowing Blood to Stand before Centrifuging.*

All sera for pH determinations were obtained from blood which had been allowed to clot at room temperature for about 10 minutes before centrifuging. It seemed possible that serum separated after longer contact with the corpuscles, especially if the blood was kept at high temperatures, might contain certain substances which would exert a disturbing influence on the quinhydrone electrode. To test this possibility, one sample of blood was drawn into cold centrifuge tubes, allowed to clot 10 minutes at room temperature, and the serum separated as rapidly as possible. A second sample was allowed to stand varying lengths of time both at room temperature and at 28–38°. The results of these experiments

are given in Table VI. In four cases the quinhydrone correction rose. The incidence of glycolysis can be plainly seen upon comparing the pH of the sera obtained by the routine method with the

TABLE VI.

*Increase in Quinhydrone Correction of Serum When Blood Is Allowed to Stand at High Temperatures before Centrifuging.*

| Dog No. | Date. | $pH_{H_2}$ | $pH_Q$ | $pH_{H_2} - pH_Q$ | Treatment of blood. |
|---|---|---|---|---|---|
| | *1929* | | | | |
| 11 | Nov. 15 | 7.410 | 7.36 | 0.05 | Kept at 22° for 10 min. to permit clotting, then centrifuged. |
| | | 7.385 | 7.31 | 0.08 | Kept at 28° for 25 min., then centrifuged. |
| 13 | Nov. 22 | 7.369 | 7.30 | 0.07 | Kept at 22° for 10 min. to permit clotting, then centrifuged. |
| | | 7.040 | 6.92 | 0.12 | Kept at 30° for 1¾ hrs.; centrifuged but serum not drawn from clot for 2 hrs. |
| 12 | Dec. 13 | 7.384 | 7.32 | 0.06 | Kept at 25° for 10 min. to permit clotting, then centrifuged. |
| | | 7.355 | 7.26 | 0.10 | Kept at 29° for 18 min., at 25° for 1 hr., then centrifuged. |
| | *1930* | | | | |
| 14 | Jan. 11 | 7.262 | 7.23 | 0.03 | Kept at 25° for 39 min., then centrifuged. |
| | | 7.257 | 7.22 | 0.04 | Kept at 30° for 18 min., at 25° for 1½ hrs., then centrifuged. |
| 11 | Jan. 16 | 7.368 | 7.31 | 0.06 | Kept at 20° for 10 min. to permit clotting, then centrifuged. |
| | | 7.335 | 7.26 | 0.07 | Kept at 25° for 1 hr., then centrifuged. |
| 15 | Jan. 24 | 7.358 | 7.34 | 0.02 | Kept at 20° for 10 min. to permit clotting, then centrifuged. |
| | | 7.264 | 7.25 | 0.01 | Kept at 38° for 3 hrs., then centrifuged. |
| 19 | Mar. 10 | 7.385 | 7.34 | 0.05 | Kept on ice for 3 hrs., then centrifuged. |
| | | 7.288 | 7.21 | 0.08 | Kept at 38° for 3 hrs., then centrifuged. |

pH of the sera which had been in contact with the clot for longer periods of time. Whereas in the case of Dog 13 the greatest increase in acidity seemed to be associated with the greatest rise in the *Q* correction, yet in Dog 15 an increase in acidity of 0.1 pH

occurred with no change in the $Q$ correction. In Dogs 11, 12, and 11 (repeated) increases in the $Q$ correction were accompanied by only small increases in acidity. Thus, there appears to be little correlation between glycolysis and the increase in the $Q$ correction. It is interesting to note that three experiments showed no change in the $Q$ correction, even though in one of these, Dog 15, the blood stood 3 hours at 38° before centrifuging.

Thus it appears that serum for pH work with the quinhydrone electrode should be separated as soon as possible from the corpuscles. While blood is usually centrifuged promptly, these

TABLE VII.

*Comparative pH Determination on Dog Serum and Plasma at 38° by Hydrogen and Quinhydrone Electrodes.*

| | $pH_{H_2}$ | $pH_Q$ | $pH_{H_2} - pH_Q$ |
|---|---|---|---|
| Serum | 7.381* | 7.36 | +0.02 |
| Plasma† | 7.422 | 7.39 | +0.03 |
| Serum | 7.384 | 7.35 | +0.03 |
| Plasma | 7.390 | 7.36 | +0.03 |
| Serum | 7.353 | 7.34 | +0.01 |
| Plasma | 7.357 | 7.34 | +0.02 |

* The animal struggled during the collection of this serum sample.

† Anticoagulant: 0.4 per cent mixture 3:1 potassium oxalate-sodium fluoride.

results suggest that the equilibration of blood samples outside the body at 38° requiring considerable lengths of time may cause changes in the serum which may influence the $Q$ correction.

*Serum and Plasma.*

The experiments listed in Table VII were performed to determine whether the $Q$ corrections for plasma are the same as for serum. Such was found to be the case.

Incidentally, the last two experiments on the pH of serum and plasma lend further substantiation to the observations of Ross (18) and Bennett (19), who found no difference in pH between serum and plasma. This is not, however, in agreement with Kugelmass

(20) nor with Stuber and Lang (21), who found that clotting produced an alkalinity due to absorption of hydrogen ions by the clot. But it must be pointed out that these latter investigators studied the mechanism of clotting with more or less purified mixtures. Their findings may not necessarily be strictly applicable to such complex systems as whole blood.

*First Acid Change.*

Recently, Havard and Kerridge (22) have published a series of determinations of the pH of human whole blood with the glass electrode. They made the very interesting and important observation that blood, after being drawn, became 0.02 to 0.05 pH more acid within 2 to 8 minutes if kept at 38°. At lower temperatures, the change came on more slowly: in 6 to 14 minutes at 28°; in 1½ hours at 18°. This acid change seemed not to be associated with clotting, since it occurred both before and after the blood clotted. Immediate chilling prevented the acid change altogether. The addition of NaF, while effective in preventing the later acid change observed by Evans (23), was non-effective. The acid change did not occur in separated serum or plasma, but did occur when corpuscles were added. Thus it appeared that the first acid change was due to an acid substance from the corpuscles. Preliminary experiments indicated that this acid substance was probably not lactic acid.

In view of our experience with blood kept at high temperatures before centrifuging, there was the possibility that the high $Q$ corrections thus obtained might reflect the presence of disturbing substances produced from the corpuscles. With this in mind, a series of determinations was made to test the findings of Havard and Kerridge, with the hydrogen and quinhydrone electrodes, and to see whether there was any correlation between the first acid change and high $Q$ corrections. It was found desirable in these experiments to use anticoagulants, since the low temperatures prolonged the clotting time of blood so greatly that the serum often clotted after centrifuging. One sample of blood was drawn into chilled centrifuge tubes kept in an ice bath; another sample was drawn into warm tubes kept at 38°. After standing at 30–35° for from 10 to 15 minutes, the warm sample was chilled, and both samples were centrifuged in the cold room. By this procedure

there was obtained a plasma sample from blood before and after the supposed first acid change. In every case, the pH of the plasma from the chilled blood was higher than that from the warmed blood. The data are given in Table VIII.

Examination of the $Q$ corrections shows that the appearance of the first acid change has left them unaltered. The relatively shorter exposures of the blood to high temperatures before centrifuging and the use of anticoagulants seem to offer a possible explanation why in this series of experiments the $Q$ corrections in the warm samples did not rise as they did in the earlier experiments of Table IV.

TABLE VIII.

*First Acid Change in Shed Blood.*

| $pH_{H^2}$ | First acid change. | $pH_Q$ | $pH_{H_2} - pH_Q$ | Treatment of blood. |
|---|---|---|---|---|
| 7.432 | | 7.44 | −0.01 | Chilled and centrifuged at 10°. |
| 7.392 | 0.040 | 7.41 | −0.02 | Kept 13 min. at 36°, then chilled and centrifuged at 10°. |
| 7.452 | | 7.44 | +0.01 | Chilled and centrifuged at 10°. |
| 7.432 | 0.020 | 7.41 | +0.02 | Kept 12 min. at 33°, then chilled and centrifuged at 10°. |
| 7.480 | | 7.47 | +0.01 | Chilled and centrifuged at 10°. |
| 7.448 | 0.032 | 7.44 | +0.01 | Kept 15 min. at 30°, then chilled and centrifuged at 10°. |

*Quinhydrone Determinations at 20°.*

Comparisons by other investigators of the pH of serum at 20° by the hydrogen and quinhydrone electrodes have been confined largely to human material. A search through the literature has revealed only two references dealing with comparative determinations on dog serum at 20°. Cullen and Earle (9) report two experiments where the quinhydrone electrode averaged 0.09 pH more acid than the hydrogen electrode value. This is somewhat higher than the correction factor they found for human serum (0.06). They state, however, that in view of the discrepancies of the colorimetric method which occur in dog serum, more data must be accumulated before such a $Q$ correction can be accurately known. The average of four values on one sample of dog plasma from Vellinger and Roche's (24) paper, gives a $Q$ correction of 0.11 pH.

In order to accumulate additional data on the $Q$ correction of dog serum at 20° and especially to enable us to compare our results with the data in the literature at room temperature, the pH of a series of dog sera was determined by the hydrogen and quinhydrone electrodes at 20°. It was found that the drift of the quinhydrone electrode was considerably less at 20°. Except in a few cases, the drift was not apparent until about 30 seconds after contact was made, thereafter, a drift toward more acid pH slowly began. As far as can be gathered from the work of Cullen and Earle, the drifts observed by them at 20° were similar to ours. Table IX shows the results obtained. The average $Q$ correction was +0.03, which is in exact agreement with our value at 38°. Save for one quin-

TABLE IX.

*Comparison of pH of Normal Dog Serum at 20° by Hydrogen and Quinhydrone Electrodes.*

| Dog No. | Date. | Hydrogen electrode. | | Quinhydrone electrode. | | $pH_{H_2} - pH_Q$ |
|---|---|---|---|---|---|---|
| | | Temperature. | pH | Temperature. | pH | |
| | *1930* | °C. | | °C. | | |
| 11 | Mar. 19 | 20.5 | 7.522 | 20.5 | 7.51 | +0.01 |
| 12 | " 22 | 20.5 | 7.598 | 19.5 | 7.59 | +0.01 |
| 16 | " 27 | 20.0 | 7.582 | 19.5 | 7.54 | +0.04 |
| 15 | " 24 | 21.0 | 7.578 | 21.0 | 7.54 | +0.04 |
| 17 | " 28 | 20.0 | 7.562 | 19.5 | 7.54 | +0.02 |

hydrone result obtained 24 hours after hemorrhage, the deviations from this mean $Q$ correction were somewhat less than those found at 38°. This may, however, not be significant in view of the comparatively small number of determinations made. It is interesting to note that except for the one case already mentioned the $Q$ corrections obtained were very much less than either those of Cullen and Earle or Vellinger and Roche.

*Temperature Coefficient.*

In four experiments given in Table X, simultaneous determinations were made at 20° and 38° on serum. From these data, the temperature coefficient of serum could be calculated on the assumption that between 20–38°, the change in pH per degree was con-

stant. The particular fact to be brought out is that this change appears to be the same for the hydrogen and quinhydrone electrodes, a conclusion which necessarily follows from the observations made concerning the $Q$ correction at 20° and at 38°. This coefficient, averaging 0.013 pH per degree in the four cases tabulated, agrees with the findings of Cullen, Keeler, and Robinson (25). A calculation from four of their experiments with the hydrogen electrode at 20° and 38° gives an average coefficient of 0.012 pH. The experiment on Dog 17 was designed to show

TABLE X.

*Temperature Coefficient of pH of Dog Serum As Determined by Hydrogen and Quinhydrone Electrodes.*

| Dog No. | Date. | Hydrogen electrode. | | | Quinhydrone electrode. | | | $pH_{H_2} - pH_Q$ |
|---|---|---|---|---|---|---|---|---|
| | | Temperature. | pH | Coefficient. | Temperature. | pH | Coefficient. | |
| | 1930 | °C. | | $\frac{dpH}{dt}$ | °C. | | $\frac{dpH}{dt}$ | |
| 15 | Jan. 29 | 38 | 7.369 | | 38 | 7.35 | | +0.02 |
| | | 22 | 7.610 | 0.015 | 21 | 7.58 | 0.014 | +0.03 |
| 11 | Apr. 4 | 38 | 7.365 | | 38 | 7.31 | | +0.06 |
| | | 21 | 7.592 | 0.013 | 20 | 7.55 | 0.014 | +0.04 |
| 17 | Apr. 17 | 38 | 7.339 | | 38 | 7.31 | | +0.03 |
| | | 20.3 | 7.582 | 0.014 | 20.2 | 7.54 | 0.013 | +0.04 |
| 17* | Apr. 18 | 38 | 7.395 | | 38 | 7.35 | | +0.05 |
| | | 20.1 | 7.623 | 0.013 | 20 | 7.55 | 0.011 | +0 07 |

* Sample taken 24 hours after a 3.7 per cent hemorrhage.

whether experimental variation of the protein concentration of serum would exercise any change on the temperature coefficient of the pH as measured with the hydrogen and quinhydrone electrodes. On theoretical grounds, a lowering of the temperature coefficient might be expected 24 hours after hemorrhage, when the serum proteins were probably somewhat diluted. Our experiment, however, does not show any decisive lowering of the temperature coefficient of the pH of the serum after hemorrhage, at least with the hydrogen electrode, and in the absence of additional experiments, the result with the quinhydrone electrode must await explanation.

*Abnormal Sera.*

During the course of the work, several serum samples were obtained from dogs which did not appear to be normal. Two samples were taken from dogs with biliary fistulas, and had the characteristic deep yellow color. These gave $Q$ corrections of $+0.07$ and $+0.05$. It may be that the presence of excessive amounts of biliary substances in the serum caused the slightly higher $Q$ correction, such as was noted in the one case. Two other samples of serum were obtained from dogs with symptoms of distemper. Here the $Q$ correction averaged $+0.05$ pH which is within the normal range. One sample of serum obtained from a dog after acid injection was very much hemolyzed. It was expected, in view of the observations of the alkaline drift of the quinhydrone electrode in whole blood (16, 26), that the drift in this particular sample would be modified. This was not the case. The drift was of the same order and in the same direction as with unhemolyzed serum. This was also our experience in several cases where only slight hemolysis occurred. Mislowitzer (27) states specifically that the presence of hemoglobin in serum does not affect the drift of the quinhydrone electrode. A buffer solution to which he added hemoglobin did not produce any drift in the quinhydrone electrode towards more alkaline values. We have, however, to explain the fact that in our hemolyzed serum, the $Q$ correction was negative instead of positive ($-0.05$). Since diluted whole blood, as Mislowitzer and also Liu (28) found, gives quinhydrone readings which average 0.02 pH more alkaline than the hydrogen electrode, this observation may explain the above negative correction. Nevertheless, in several samples of serum which were only slightly hemolyzed no significant changes in the $Q$ corrections could be observed.

DISCUSSION.

The following facts were gleaned from a study of the distribution curve of the $Q$ corrections of 65 determinations, which include all the serum and plasma, normal and abnormal. Out of this number it seems reasonable to eliminate the very small number of extreme variations of the $Q$ corrections, especially when one remembers that the causes which are responsible for their occurrence appear to be known and can, therefore, be avoided. When this is done, the

limits of the $Q$ correction are from $-0.02$ to $+0.06$, a spread of 0.08 pH. If the average $Q$ correction is taken as $+0.03$, then within these limits 93 per cent of all the determinations checks results with the hydrogen electrode within $\pm 0.03$ pH and 83 per cent within $\pm 0.02$. The variations in the $Q$ correction do not seem to have any relation to the pH of the serum.

A sufficiently large number of comparative determinations of the pH of dog serum and plasma have been made under a variety of experimental conditions to justify an evaluation of the quinhydrone method. Since the electrode represents an oxidation-reduction system, undesirable side reactions with some of the constituents of the solutions in which it is to be used may take place. This may produce drifting potentials. In serum at 38° the drift is so great that it is necessary to resort to extrapolation to obtain suitable results. On theoretical grounds, such a procedure is open to serious criticism; on practical grounds, the increased complexity of technique and consequent possibility of error are important considerations. The method is empirical; the accuracy of its results must largely serve as its justification. But it is believed that a degree of accuracy is attainable which is adequate for much of the present biochemical research. The drifts are far less at 20°, and the determination is, therefore, simpler, but at the present time we hesitate to assume that the temperature correction which must be applied to obtain values for 38° is constant for all serum.

This study indicates that the method of determining pH by the quinhydrone electrode is subject to fewer errors than the colorimetric method. It possesses no inherent subjective errors, and in general gives more reliable data under experimental conditions. While less accurate than the hydrogen electrode, the small amounts of material needed for the quinhydrone electrode determination, and the rapidity with which determinations may be made, make the quinhydrone electrode a decidedly useful instrument for studying the pH of blood and other tissue fluids.

SUMMARY.

1. A comparative study of the pH of normal dog serum and plasma at 38° by the hydrogen and quinhydrone electrodes has been made. On the average, the quinhydrone electrode reads 0.03 pH more acid than the hydrogen electrode. Under experi-

mental conditions this difference has been found to remain sufficiently constant to inspire confidence in the reliability of the method. Determinations of pH with the quinhydrone electrode can be rapidly made and require but small amounts of fluid.

2. The occurrence of the first acid change of shed blood, discovered by Havard and Kerridge, has been substantiated both with the hydrogen and quinhydrone electrodes.

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